PB85-167914

Evaluation of a Site-Specific Criterion Using Outdoor Experimental Streams

(U.S.) Environmental Research Lab.-Duluth
Monticello, MN

Feb 85

U.S. Department of Commerce
National Technical Information Service
NTIS

PB85-167914

EPA/600/D-85/041
February 1985

EVALUATION OF A SITE-SPECIFIC CRITERION USING OUTDOOR EXPERIMENTAL STREAMS

by

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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA/600/D-85/041		

4. TITLE AND SUBTITLE	5. REPORT DATE
Evaluation of a Site-Specific Criterion Using Outdoor Experimental Streams	February 1985
	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
S. F. Hedtke and J. W. Arthur	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
U.S. Environmental Protection Agency Monticello Ecological Research Station P.O. Box 500 Monticello, MN	11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Environmental Research Laboratory U.S. Environmental Protection Agency Office of Research and Development Duluth, MN	14. SPONSORING AGENCY CODE EPA-600/03

15. SUPPLEMENTARY NOTES

16. ABSTRACT

An overview of a study to evaluate a site-specific water quality criteria for pentachlorophenol (PCP) in outdoor experimental streams is presented. The site-specific criterion was calculated from acute toxicity results for eight resident species and the relationship between acute and chronic toxicity of PCP. The PCP concentration expected to protect aquatic life (30-day average criterion concentration) was less than or equal to 48 µg/L. Outdoor experimental streams were subsequently dosed continuously for 84 days at 48, 144, and 432 µg PCP/L. Measurements of the biological structure and ecosystem processes within the exposure streams were compared to a control system. Effects on snails were found only at 432 µg/L, but effects on fish, periphyton, and system metabolism were found at 432, 144, and 48 µg/L. The small differences between the criterion-dosed stream (48 µg/L) and the control stream may have been caused by PCP or interstream variation. The acceptibility of this criterion concentration depends in part on the definition of protection of aquatic life and its uses.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to public	Unclassified	25
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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INTRODUCTION

An important application of aquatic toxicological data has been the development of water quality criteria. These criteria are intended to be the highest concentrations of individual pollutants which will not adversely affect aquatic life and form the scientific basis for the establishment of water quality standards. Both the methods of derivation and resulting numerical values have been periodically reevaluated and adjusted to incorporate new data. The Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Life and Its Uses (National Guidelines) [1] propose a two-number criterion: a maximum concentration and a 30-day average concentration. The average concentration can only be exceeded for up to 96 hours during any 30-day period. The maximum concentration is based on the results of acute toxicity tests with aquatic animals whereas the average concentration is derived from chronic toxicity tests, bioaccumulation, and any additional effects information. However, due to differences in water quality and biological species composition among aquatic systems, the national criteria may not be applicable to specific locations. Therefore, developing site-specific criteria has been suggested as a means to more accurately deriving criteria which should protect a particular water-body of interest. Several procedures [2] have been developed for taking into account site-to-site variations in water quality and species composition.

Although site-specific toxicity results account for variations in site water and species composition, the site-specific guidelines are still based on results of laboratory tests and the applicability of this information to natural systems needs evaluation. Two basic questions are involved in this comparison. The first is whether species are affected similarly in laboratory

tests and field situations. Factors in the field which might alter the effect include exposure conditions, life stages, predation, competition, nutritional value of food resources, disease, and others [3, 4]. Because resident species are used in the the development of site-specific criteria, comparisons of identical species can be made. However, it may not be possible to acceptably measure similar endpoints in both laboratory and field situations. For example, effects on reproduction of aquatic insects are difficult to measure in the lab whereas effects on cladoceran reproduction are difficult to measure in the field. Thus, direct comparisons of effects may not always be possible. The second question is whether guidelines for deriving criteria result in concentrations that protect aquatic life and its uses. Regardless of whether individual species are similarly affected in laboratory and field situations, criteria may or may not be appropriate. To address this issue both structural and functional properties of the system should be studied.

The primary objective of this study was to evaluate the applicability of a laboratory-derived site-specific criteria for a representative material in an outdoor aquatic ecosystem. The field evaluation utilized measurements of ecosystem characteristics including energy fixation, material cycling, and biological structure. There were also several secondary objectives including the following:

1. To compare effects on individual species in laboratory and field situations both in terms of prediction of effects and in the relative ranking of the sensitivity of species.

2. To evaluate the relative sensitivity of effects on species compared to functional properties.

3. To determine improvements needed in the design of field evaluations of the effect of test materials on aquatic ecosystems.

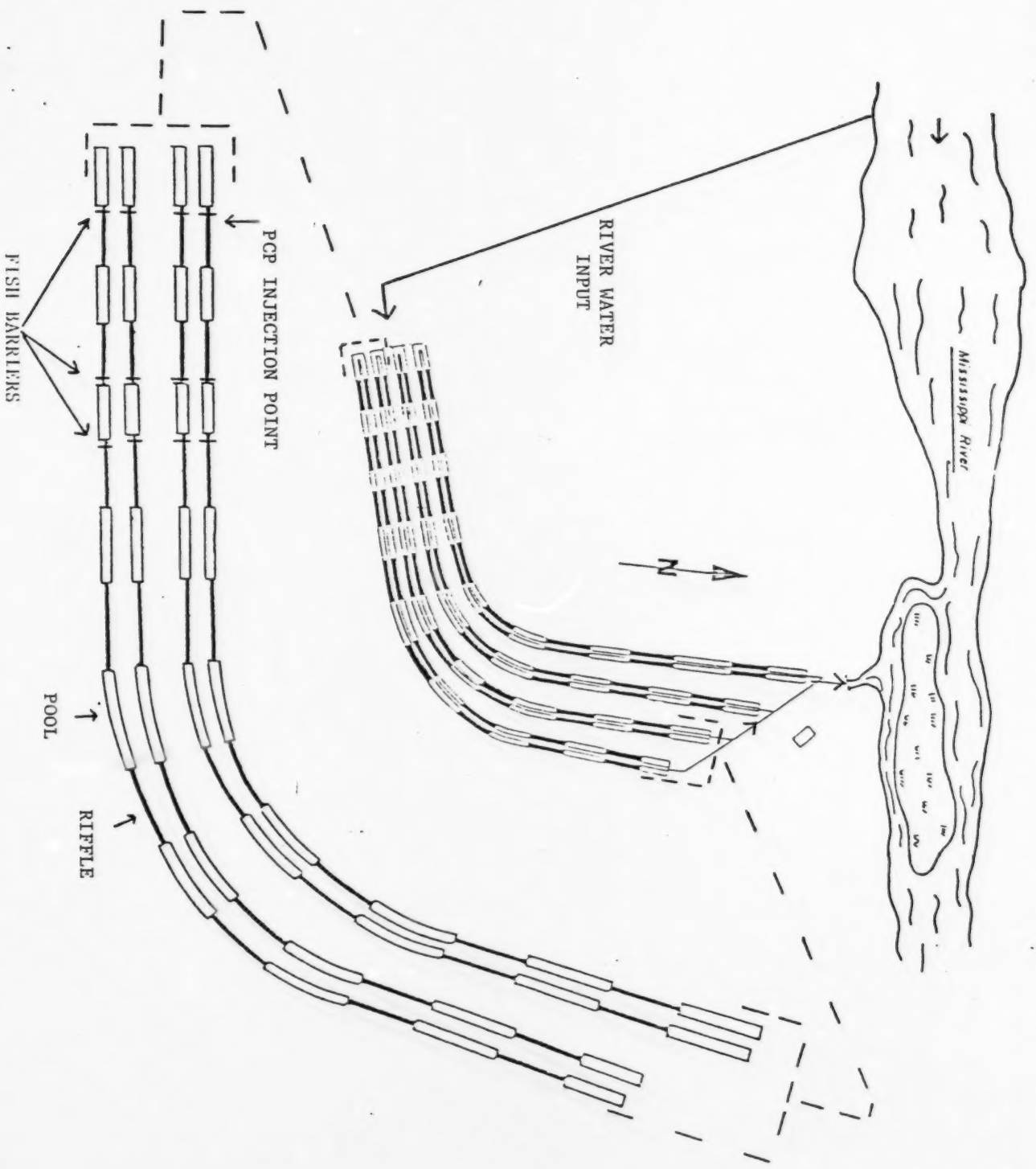
The purpose of this paper is to present an overview of a study using pentachlorophenol. Details of this project will be published elsewhere.

METHODS

The material selected for study was pentachlorophenol (PCP), a widely used algicide, fungicide, herbicide, and insecticide. Dowicide EC-7 (93.7% PCP) was the formulation used for both laboratory and field tests. The latest U.S. EPA criteria document for PCP [5] did not propose a criteria but stated that available data indicates that acute toxicity, based on species mean acutes, occurs as low as 55 and chronic toxicity, based on 10% salmonid growth inhibition, occurs as low as 3.2 µg/ℓ. Acute toxicity values for freshwater organisms ranged from 34 to 2000 µg/ℓ. Available chronic toxicity values were 240 µg/ℓ for the cladoceran, Daphnia magna, and 57 µg/ℓ for the fathead minnow, Pimephales promelas. The toxicity of PCP also has been shown to be influenced by environmental conditions such as pH [6, Robert Spehar, personal communication] and temperature [7, 8, 9]. In addition, PCP degrades photolytically [10] which can result in concentration variations in natural systems over a normal day-night period.

The outdoor aquatic ecosystems used for the field tests were the experimental streams at the U.S. EPA Monticello Ecological Research Station (MERS), a field station of the Environmental Research Laboratory-Duluth (ERL-D). This station is located on the Mississippi River approximately 70 km northwest of Minnespolis, MN. Each of the eight experimental streams available at this site [11, 12] are 520 m long with 30.5 m alternate mud-bottom and gravel riffles (Figure 1). The pools are 3.4 to 3.5 m wide and 0.7 to 0.8 m deep; riffles are 1.2 to 1.3 m wide and 0.1 to 0.16 m deep. Water is supplied directly from the Mississippi River. Plants in the streams consist of Elodea canadensis, Potamogeton crispus, Ceratophyllum demersum and Lemna minor as well as numerous algal species. The invertebrate community in the streams is

Figure 1. Configuration of Outdoor Experimental Streams



the result of natural colonization. Fish are introduced each year as desired.

Laboratory Tests

A site-specific criterion for PCP was calculated according to procedures currently described in the Guidelines for Deriving Numerical Site-Specific Water Quality Criteria for the Protection of Aquatic Life and Its Uses [2]. The resident species approach was utilized as follows:

1. Flow-through acute toxicity tests were conducted in Mississippi River water under ambient conditions with two fish species and six invertebrate species using ASTM [13] procedures. These species are the dominant macroinvertebrates and the two fishes stocked in the experimental streams. Fish were wild stock collected from local lakes and macroinvertebrates were collected from unexposed experimental streams.

2. A site-specific Final Acute Value was calculated from these data according to the method described in the National Guidelines. This value is an estimate of the toxicant concentration corresponding to a cumulative proportion of 0.05 in the acute toxicity values for the families with which acute tests have been conducted. Because data were available for only 8 families, the Final Acute Value was lower than the measured LC50 values for any of the tested families.

3. An acute-chronic ratio was calculated from life-cycle and early-life stage tests conducted at ERL-D in Lake Superior water.

4. The site-specific Final Chronic Value was calculated by dividing the Final Acute Value by the acute-chronic ratio.

5. Final Residue and Plant Values were not calculated because relevant data concerning the significance of PCP residues and site-specific plant

effects were not available. However, subsequent plant tests indicated that macrophytes were less sensitive than organisms tested as part of the criterion development.

6. The site-specific Final Chronic Value was, therefore, used as the 30-day average concentration. The resident species approach is essentially a direct application of the National Guidelines to a specific site and therefore incorporates the assumptions included in these guidelines.

Field Study

Four of the experimental streams were used for this study (Figure 1). The initial 30.5 m pool in each stream was used a a settling basin for incoming suspended solids. The next 122 m, including two pools and two riffles, was used as the experimental area. Water was supplied to each stream at a rate of 0.76 m^3/min (200 gal/min). Three stainless steel mesh barriers were placed in each stream to separate and confine adult fish in the experimental study area: one at the head, one at the end of the second riffle, and one at the end of the second pool, respectively. A series of A-frame wooden shade structures were positioned over the first study pool to reduce aquatic plant growth. The total available light under the shade structure was reduced by 70-75 percent. Wild fathead minnows, Pimephales promelas, were stocked in the first experimental pool and bluegills, Lepomis macrochirus, in the second pool.

PCP was added to three of the streams in the form of dissolved sodium pentachlorophenate while the fourth stream served as a control. Concentrations selected were the 30-d average concentration, three times the criterion, and nine times the criterion. Continuous metered volumes were added to the

streams at the head of the first riffle. Dosing continued for 84 days from 3 June 1982 to 26 August 1982. PCP concentrations were determined by gas chromatography following hexane extraction and formation of the ethoxy derivative with freshly prepared diazoethane. Samples for analyses were collected routinely throughout the experimental reach and occasionally at the end of each of the streams. Comparisons of nighttime and daytime concentrations were also conducted monthly.

To measure the effect of PCP on the experimental streams, measurements of both biological structure and function were made. A listing of the structural components, functional properties and measurement procedures is presented in Table 1. These responses were representative of the important structural and functional characteristics of the experimental streams. It was not possible to study all the components and functions of the streams, so a limited number was selected based on scientific judgement of importance and expected sensitivity, available resources, and the state-of-the-art of field measurement techniques. Because toxicity predictions as well as field evaluations usually are based on species, structural components were emphasized. Although selected functional properties were measured, measures of some important aspects such as nutrient cycling were not conducted due to resource limitations.

Table 1. Biological Measurements in Outdoor Streams.

Structural Components	Measurements	Method
Bacteria	Total counts	Acridine orange direct count
	PCP degraders	^{14}C-PCP most probable number
Zooplankton	Dominant cladoceran species	Qualitative horizontal tow
Macroinvertebrates	Taxa and numbers	Submerged substrates
	Drift	Nets
	Snail reproduction	Egg mass counts on substrates
Fish - fathead minnows and bluegill sunfish	Survival	Final number by seining
	Growth: weight, length	Traps
	Reproduction: fecundity	Egg counts on substrates/nests
	Drift: numbers	Nets
Phytoplankton	Taxa and numbers	Grab samples
	Chlorophyll *a*	Grab samples
Periphyton	Species, biomass	Substrate colonization
	ATP, chlorophyll *a*	

Functional Measurements	Measurement	Method
Litter decomposition	Weight loss	Litter bags
Sediment methanogenesis	Methane production	Laboratory incubation
System metabolism	Diel range of pH and D.O.	Continuous monitoring with instream probes
PCP degradation	Change in [PCP]	Chemical analyses

RESULTS

Laboratory Tests

The eight 96-hr LC50 values in Mississippi River water ranged from 200 to 2300 µg/ℓ (Table 2). Fish were the most sensitive of the tested organisms with fathead minnows and bluegills being equally sensitive. Physid snails and cladocerans were the most sensitive invertebrates. Due to the rapidly changing water characteristics of the Mississippi River in the spring, the temperature, pH, and dissolved oxygen concentration of these tests ranged from 7.3 to 17.4°C, 7.5 to 8.2 units, and 7.7 to 12.2 mg/1, respectively. Acute and chronic tests conducted at ERL-D resulted in an acute-chronic ratio of 2 (Donald Mount, personal communication). Division of the Final Acute Value by the acute-chronic ration resulted in a calculated site-specific 30-day average concentration of 48 µg PCP/ℓ.

Field Studies

The experimental streams were dosed at 48 µg/ℓ, 144 µg/ℓ, 432 µg/ℓ. Based on the laboratory toxicity results, predictions of stream effects were as follows:

1. Fathead minnows and bluegills would die at 432 µg/ℓ.
2. Sub-lethal effects on fish would be observed at 144 µg/ℓ.
3. No effects on fish would be observed at 48 µg/ℓ.
4. Physid snails and cladocerans would be impacted at 432 µg/ℓ.
5. No effects on invertebrates would be seen at 48 and 144 µg/ℓ.

Predictions of specific effects on organisms other than those actually tested in the laboratory could not be made directly. However, because the objective of criteria derivation is to protect aquatic ecosystems, it was expected that the stream exposed to 48 µg/ℓ PCP would not be unacceptably affected.

Table 2. Results of Acute Toxicity Tests with PCP in Mississippi River Water.

Rank	Species	LC50* (µg/ℓ)	Confidence Interval
1	Bluegill *Lepomis macrochirus*	200	190-220
2	Fathead minnow, *Pimephales promelas*	200	160-300
3	Cladoceran, *Simocephalus vetulus*	670	-
4	Snail, *Physa gyrina*	730	710-750
5	Caddisfly, (species as yet undetermined)	1200	1150-1390
6	Mayfly, *Callibaetis skokianus*	1700	1270-2480
7	Amphipod, *Crangonyx pseudogracilis*	1900	1420-2510
8	Isopod, *Asellus racovitzai*	2300	1550-3600

Final Acute Value = 96 µg PCP/ℓ

*96-hr except for cladocera = 48-hr

Although the input of PCP was constant, the concentrations of PCP decreased both from the beginning to the end of each stream and from the beginning to the end of the study at points within each stream. By the completion of the study the concentrations at the ends of the experimental areas were approximately 70% of input for the 48 µg/ℓ criterion stream, 50% of the input for the 144 µg/ℓ stream, and 55% of input for the 432 µg/ℓ stream. In addition, diel fluctuations in PCP concentrations within the experimental areas resulted in daytime concentrations as much as 40% less than nighttime values due to photolysis.

The biological field results are summarized in Table 3. The determination of differences from the control stream were based on the professional judgements of the principal investigators for each component and statistical analyses where possible. Generally, statistical analysis was not possible and judgements of effects were based on dose-response relationships. Therefore, a gradation of differences from slight to dramatic is indicated. The most dramatic effect was complete fish mortality at 432 µg/ℓ. Slight or potential effects were those where a dose-response relationship was observed but differences from controls (usually >10%) may not have been biologically significant. For example, growth of female fatheads at 48 µg/ℓ was consistently, and occasionally statistically, lower than in the control but represented only a 10-15 percent reduction in mean weight.

Table 3. Summary of Biological Differences Between Control and Exposed Streams.

Biological Component	Nominal PCP Concentration (µg/ℓ) 48	144	432
1. Structure			
Bacteria - total	0[a]	0	0
- PCP degraders	++	+++	+++
Zooplankton	0	+	+
Macroinvertebrates - total abundance	0	0	0
- species diversity	0	0	0
- drift	0	0	++
- snail abundance	0	0	++
- snail reproduction	0	0	+++
Fish - survival	0	0	++++
- growth	+	++	++++
- reproduction	0	++	++++
- larval drift	+	+	++++
Phytoplankton - total abundance	0	0	0
- species distribution	0	0	0
- chlorophyll	0	0	0
Periphyton - species	+	+	+++
- biomass	+	++	+++
- ATP	+	++	+++
- chlorophyll	+	++	+++
2. Function			
Litter decomposition - cattail	0	0	0
- red maple	0	0	0
Sediment methanogenesis	-[b]	-	-
System metabolism	+	++	+++
PCP degradation	++	+++	++++

[a] 0 = no difference from control
+, ++, +++, ++++ = gradation of differences from control ranging from slight to dramatic

[b] - = No difference from control, pending TOC standardization of channel sediments

DISCUSSION

Comparisons between laboratory and field effects of materials are frequently limited by the ability to detect effects in the field. Biological differences between exposed and control streams may occur due to the effects of experimental manipulation and/or natural variations. Severe effects such as death of fish is convincing evidence of chemical effects. In addition, consistent concentration-effect relationships suggest that effects were principally due to the chemical. However, as the differences between control and exposed streams become less obvious, natural variations become more important. Natural variations among ecosystems, even those apparently physically similar, is well known. Physical conditions were controlled and made as similar as possible in the present study. Nonetheless, natural pretest differences such as variations in macroinvertebrate and periphyton densities existed.

Detection of more subtle effects may require replication of exposures and/or control streams to establish normal limits of reproducibility. Such replication could take place either spatially or temporally. Spatial replication, such as is done in laboratory tests, could consist of studying several physically identical systems under the same exposure conditions at the same time. Replication of this type allows quantitative estimates of the reproducibility of measured responses and more rigorous statistical analysis. In most field situations resource and/or site limitations usually preclude such replication. Upstream control pools and riffles within each stream might be used as a reference point. Upstream-downstream comparisons, typically used in field studies [14], assume that the system is homogeneous within the length of the study site. The extent of differences that can be

attributed to a chemical depends on the extent of biological homogeneity. Separate pools and riffles within an exposed stream might also be considered as spatial replicates but these replicates are not truly statistically independent. Biological differences between otherwise similar streams are not likely to be limited to only one section of a stream. Thus, replication of pools and riffles within a stream will allow better estimates of effects within a stream but not necessarily comparisons between streams.

Temporal replication consists of repeating exposure conditions at different time intervals. However, seasonal and annual variations in meteorological conditions and water quality can influence the quantitative characteristics of the biological community. These temporal differences may be sufficiently large to prevent separation of toxicity from natural variations when making quantitative comparisons with previous years. Temporal replication of field effects therefore, may be best utilized by making comparisons of year-to-year similarity in concentration-effect relationships. Similar year-to-year relationships would suggest that the differences were due to the chemical.

Comparisons between laboratory and field effects of chemicals should also be evaluated in the context of similar exposure conditions. Laboratory tests using site water incorporate water quality variations during lab tests but cannot incorporate variations during the field study and effects of the ecosystem on exposure. In this study changes in PCP concentrations were found over a 24-hr period due to photolysis, and over the summer due to an increased rate of bacterial degradation. The diel variation in PCP resulted in higher concentrations at night coinciding with lower pH values when labor-

atory results indicate PCP is more toxic (Robert Spehar, personnal communication). In contrast, longitudinal decreases in PCP concentration due to degradation coincided with increased water temperatures when laboratory results indicate PCP is less toxic. Thus, in this case the lab to field comparison was complicated by varying concentration and toxicity in the field tests.

Despite complex exposure conditions and variations among streams, the following predictions from laboratory results agreed with field effects:

1. All fish died in the 432 µg/ℓ stream.
2. Sublethal effects on fish were found in the 144 µg/ℓ stream.
3. Physid snails were adversely impacted at 432 µg/ℓ.
4. No apparent effects on invertebrates were observed at 48 and 144 µg/ℓ.

The accuracy of species-by-species comparisons of laboratory toxicity with the field toxicity was only questioned by the possibility of adverse effects on fish in the 48 µg/ℓ stream. While there may be some question as to the quantitative prediction of sublethal effects on fish in the field from laboratory results, the order of sensitivity of species tested in the laboratory was the same as that observed in the field. The question, therefore, becomes one of trying to determine subtle differences in biological measurements between control and criterion streams. As part of the continuing evaluation of the application of laboratory-derived criteria, additional field research is underway at this laboratory to further study the significance of the effects of PCP at the criterion concentration.

Absolute cause-effect relationships for subtle differences may never be fully demonstrated under complex field conditions. Professional judgement

is, therefore, an important feature in interpreting field effects. Judgement becomes especially important in determining whether a particular concentration, i.e. criterion, actually "protects" an aquatic ecosystem. This is an important objective in evaluating national and site-specific criteria. In the present study field effects of PCP that were not be directly predicted from the laboratory tests were observed on periphyton and system metabolism. These results suggest that untested components may not have been fully protected at the criterion concentration. In addition, functional parameters in some cases may be as sensitive as structural parameters. The decision as to the acceptability of a criterion may, therefore, depend on the definition of protection as the objective of a criterion.

Historically the definition of ecosystem protection has not been clearly stated. The 1968 Water Quality Criteria [15], the so-called "Green Book", discusses criteria as a means to "... define water quality requirements which must be met to insure a favorable environment for fish, other aquatic life, and wildlife." Such wording continues in the 1976 version Quality Criteria for Water [16], the "Red Book", where criteria are to provide "for a water quality that will provide for the protection and propagation of fish and other aquatic life ... in accord with the 1983 goals of P.L. 92-500." The 1972 Federal Pollution Control Act Amendments (P.L. 92-500) calls for water capable of supporting balanced populations of aquatic life and biological integrity. The American Fisheries Society review of the Red Book [17] defines criteria as those characteristics which "... when incorporated into standards, will protect defined uses of the nation's waters with an adequate degree of safety." In each of these definitions or descriptions of criteria, the

concept of protection is stated or implied with little specifics as to what protection means to an aquatic community. Thus, the protection that is the objective of water quality criteria, is never clearly stated.

The idea that criteria may be protective for an ecosystem and yet not necessarily protective of all species within the ecosystem is an explicit feature of the National Guidelines which state that protection of all species all of the time is not necessary. The site-specific guidelines describe criteria as numerical values which "must protect most of the species nearly all of the time." The determination of what is an expected or reasonable number of affected species or the extent of acceptable effect is again left to interpretation.

An approach that might be taken is for the scientific community to describe the various degrees of risk or potential damage to aquatic life associated with various levels of pollution, A criterion would be a risk curve rather than a specific concentration. However, because such an approach is beyond current state-of-the-art, scientists should derive criteria that provide an acceptable level of protection. Society may subsequently choose to accept or modify such recommendations in the derivation of standards. Therefore, it is important for the scientific community to clearly define what level of protection is intended when criteria are recommended. Perhaps the state of the art of ecology and toxicology is such that we cannot define protection. Perhaps we as scientists are not yet at the stage where we know precisely what to protect and at what level of protection. However, if the scientific community does not define this concept, eventually regulators, legislators, or the courts may decide for us.

In summary predictions of PCP effects made from laboratory test results were generally in agreement with observed field effects. However, some results suggested that the criterion may not have completely prevented adverse effects on the aquatic community. Effects at this concentration may have been due to PCP or to interstream variations. Due to uncertainties in the objectives of water quality criteria, conclusions regarding the acceptability of the site-specific criterion must, at the present time, remain a matter of individual interpretation. A clear definition of the risks associated with criteria must be developed by the scientific community to allow appropriate translations of criteria to standards.

Acknowledgements

This study was a cooperative effort between the U.S. EPA, St. Olaf College, and the Gray Freshwater Biological Institute. Contributors of results obtained in this study include J. Pignatello and K. Nordlie Allen, chemistry; R. Hermanutz, Fish; J. Zischke and N. Larson, invertebrates; M. Martinson and R. Crawford, microbiology; D. Hogan, phytoplankton; D. Yount, periphyton; C. West, T. Norberg and D. Mount, toxicity tests; R. Andrew, productivity; T. Henry, and J. Johnson, facility and dosing; and G. Ericksen, statistics. Charles Stephan provided useful discussion and comments during the development of this paper.

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